CLAIMS

What is claimed is:

1. A primer set comprising:

(a) at least two primers capable of amplifying a portion of all human leukocyte antigen (HLA) alleles of an HLA locus; and

(b) a control primer pair capable of producing an HLA control amplicon of predetermined size by amplifying a portion of a HLA allele only if the HLA locus is present in a sample.

2. The primer set of claim 1 wherein the portion of the HLA allele amplified by the control primer pair is common to all or substantially all HLA alleles.

3. The primer set of claim 1 wherein the portion of the HLA allele amplified by the control primer pair comprises a portion of exon 4 of the HLA A locus or exon 4 of the HLA B locus.

4. The primer set of claim 1 wherein the predetermined size of the HLA control amplicon is about 500 to 1000 base pairs in length.

5. The primer set of claim 1 wherein at least one of the at least two primers has a 5′ portion that is not complementary to the HLA allele.

6. The primer set of claim 5 wherein the 5′ non-complementary portion decreases a melting temperature (Tm) between the primer and a HLA allele, further wherein the decreased melting temperature results in an enhanced specificity of an amplification reaction.

7. The primer set of claim 5 wherein the 5′ non-complementary portion allows for amplification of a more abundant product, further wherein the 5′ portion allows for a more robust amplification reaction.

8. A primer set comprising:

(a) a multiplicity of primers capable of simultaneously amplifying a plurality of a portion of Class I HLA alleles of a HLA locus under a single set of reaction conditions in a multiplex polymerase chain reaction.

9. The primer set of claim 8 wherein the plurality of a portion of Class I HLA alleles belong to a same HLA locus.

10. The primer set of claim 6 wherein the same HLA locus is a HLA A or a HLA B locus.

11. The primer set of claim 5 wherein the multiplicity of primers are capable of producing a first amplicon and a second amplicon from the HLA locus.

12. The primer set of claim 8 wherein the first amplicon spans exon 1 to intron 3 and the second amplicon spans intron 3 to exon 5.

13. The primer set of claim 8 wherein at least one of the multiplicity of primers has a 5′ portion that is not complementary to the portion of the Class I HLA allele.

14. The primer set of claim 13 wherein the 5′ non-complementary portion allows a decrease in a melting temperature (Tm) between the primer and a HLA allele, further wherein the decreased melting temperature results in an enhanced specificity of an amplification reaction.

15. The primer set of claim 13 wherein the 5′ non-complementary portion allows a more abundant product during amplification, further wherein the 5′ portion allows a more robust amplification reaction.

16. A primer for sequencing an HLA allele comprising:

(a) a primer comprising a 3′ portion and a 5′ portion wherein the 3′ portion is complementary to an HLA allele and the 5′ portion is not complementary to the HLA allele, wherein the primer allows complete resolution of an exonic sequence by a sequencing reaction.

17. The primer of claim 16 wherein the 5′ non-complementary portion is 1 to about 35 bases.

18. The primer of claim 16 wherein the primer allows complete resolution for one of exon 2 or exon 3 in an allele of the HLA B locus.

19. The primer of claim 16 wherein the primer allows complete resolution of exon 1 in an allele of the HLA B locus.

20. The primer of claim 16 further comprising at least one additional primer complementary to a different HLA allele.

21. The primer of claim 16 wherein the 5′ non-complementary portion allows a single electrophoresis gel to be used for all sequencing products.

22. The primer set of claim 16 wherein the 5′ non-complementary portion allows a decrease in a melting temperature (Tm) between the primer and a HLA allele, further wherein the decreased melting temperature results in an enhanced specificity of a sequencing reaction.

23. The primer set of claim 16 wherein the 5′ non-complementary portion allows a more abundant product during sequencing, further wherein the 5′ portion allows a more robust sequencing reaction.

24. A primer set comprising:

(a) a multiplicity of primers capable of simultaneously sequencing a plurality of HLA alleles of a HLA locus under a single set of reaction conditions in a multiplex sequencing reaction.

25. The primer set of claim 24 wherein the plurality of HLA alleles is a plurality of a portion of HLA alleles.

26. The primer set of claim 24 wherein the HLA locus comprises all loci of HLA Class I.

27. The primer set of claim 24 wherein the HLA locus comprises all loci of HLA Class II.

28. The primer set of claim 24 wherein the HLA locus comprises all loci of DRB.

29. A method for amplifying a class I HLA allele comprising:

(a) performing an amplification reaction on a sample having or suspected of having a Class I HLA allele wherein the amplification reaction utilizes the primer set of claim 8.

30. The method of claim 29 further comprising sequencing any resulting HLA amplicons.

31. The method of claim 29 wherein the sample is a cDNA.

32. A method for detecting the presence of an HLA allele comprising:

(a) amplifying a nucleic acid wherein the amplification reaction comprises at least two primers capable of amplifying all HLA alleles of an HLA locus and a control primer pair capable of producing an HLA control amplicon of predetermined by amplifying a portion of a HLA allele only if the HLA locus is present in the sample; and

(b) detecting the presence of the HLA allele.

33. The method of claim 32 wherein the portion of the HLA allele amplified by the control primer pair is common to all or substantially all HLA alleles.

34. The method of claim 33 wherein the portion of the HLA allele amplified by the control primer pair comprises a portion of exon 4 of the HLA A locus or exon 4 of the HLA B locus.

35. The method of claim 32 wherein predetermined size of the HLA control amplicon is about 500 to 2200 base pairs in length.

36. The method of claim 32 wherein the nucleic acid is a cDNA.

37. The method of claim 32 wherein detecting the presence of the HLA allele comprises whole HLA locus sequencing.

38. The method of claim 32 wherein detecting the presence of the HLA allele comprises partial HLA locus sequencing.

39. A method for isolating and amplifying an HLA allele comprising:

(a) reverse transcribing a RNA from a sample to form a cDNA; and

(b) performing an amplification reaction on the cDNA, wherein the amplification reaction utilizes the primer set of claim 8.

40. The method of claim 39 further comprising performing step (a) and step (b) simultaneously.

41. A method for amplifying and detecting the presence of an HLA allele comprising:

(a) amplifying a nucleic acid wherein the amplification reaction comprises at least three primers capable of amplifying all HLA alleles of an HLA locus in a multiplex amplification reaction; and

(b) detecting the presence of the HLA allele.

42. The method of claim 41 wherein detecting the presence of the HLA allele comprises sequencing the amplified nucleic acid in a multiplex sequencing reaction.

43. The method of claim 41 wherein step (a) and step (b) are automated.

44. The method of claim 43 further comprising automation on an array.

45. A kit for amplifying and detecting human leukocyte antigen alleles comprising:

(a) at least two primers capable of amplifying a portion of all human leukocyte antigen (HLA) alleles of an HLA locus; and a control primer pair capable of producing an HLA control amplicon of predetermined size by amplifying a portion of a HLA allele only if the HLA locus is present in a sample; and

(b) at least one primer comprising a 3′ portion and a 5′ portion wherein the 3′ portion is complementary to an HLA allele and the 5′ portion is not complementary to the HLA allele, wherein the primer allows complete resolution of an exonic sequence by a sequencing reaction.